PATENT APPLICATION

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of	Docket No: Q87778
Jaume Ribas PINOL, et al.	
Appln. No.: 10/535,416	Group Art Unit: 1645
Confirmation No.: 7473	Examiner: Khatol S SHAHNAN SHAH
Filed: May 19, 2005	

For: LIVE ATTENUATED VACCINE AGAINST PORCINE PLEUROPNEUMONIA

STATEMENT OF SUBSTANCE OF INTERVIEW

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

Please review and enter the following remarks summarizing the interview conducted on July 1, 2009:

REMARKS

An Examiner's Interview Summary Record (PTO-413) was mailed July 10, 2009.

During the interview, the following was discussed:

1. Brief description of exhibits or demonstration: Powerpoint presentation provided by Applicants and submitted with the Amendment filed concurrently.

2. Identification of claims discussed: All pending.

3. Identification of art discussed: Reimer, MacInnes, Prideaux

4. Identification of principal proposed amendments: None

5. Brief Identification of principal arguments: None

6. Indication of other pertinent matters discussed: Applicants gave a complete description of the invention and distinguished the claimed invention over the cited art.

7. Results of Interview:

Applicants thank Examiner Shannan-Shah and Supervisor Mondesi for granting the telephone interview on July 1, 2009 with Applicants' legal representatives, Ms. Susan J. Mack and Dr. Tu A. Phan, Sugranes attorney, Ms. Mathilde Triguel, inventors of Laboratorios HIPRA, Dr. Jaume Pinol and Dr. Enrique Querol, patent manager of Laboratorios HIPRA, Ms. Gloria Pujol, Research and Development representative for Laboratorios HIPRA, Dr. Marta Sitja, and European Patent Attorney, Mr. Rafael Pi. Applicants note that a copy of the presentation (submitted herewith) was provided to both Examiner Shannan-Shah and Supervisor Mondesi to facilitate discussion during the interview.[1]

During the telephone interview, Applicant's representative stated that there appeared to be a basic misunderstanding regarding the claimed invention, and that the purpose of the interview was to (1) clarify the claimed invention and (2) distinguish the claimed invention, which is an isolated immunogenic, non-haemolytic *Actinobacillus pleuropneumoniae* (*App*) strain comprising at least one mutation in the transmembrane domain-encoding segment of the *apx*IA gene and with or without at least one mutation in the transmembrane domain-encoding segment of the *apx*IIA gene, over the art cited by the Examiner.

[1] In accordance with M.P.E.P. § 609.05(c), the documents provided herein in support of Applicants' remarks are being submitted as evidence directed to an issue raised in the Official Action, and no fee pursuant to 37 C.F.R. 1.97 or 1.98, or citation on a FORM PTO/SB/08A & B is believed to be necessary.

With regard to (1), Dr. Pinol explained how the ApxI and ApxII exotoxins are synthesized from *Actinobacillus pleuropneumoniae* (APP), as well as the structure and mechanism of action of the ApxI and ApxII exotoxins. That is, the genes of ApxI and ApxII are structurally organized as operons in which the operon of ApxI exotoxin contains four genes designated as *apx*IC, *apx*IA, *apx*IB, and *apx*ID, and the operon of ApxII exotoxin contains two genes designated as *apx*IIA and *apx*IIC (slide 3 of presentation). The A gene is the structural gene of the exotoxin. The C gene codes for an acylase enzyme that activates the exotoxin post-translationally, the B and D genes are responsible for secreting the exotoxin by creating holes in the APP membranes. In order for exotoxins to exert their effect on swine target cells, the exotoxins must be exported outside of the APP in order to reach the target cells. For this reason, APP strains without the B and D genes are non-haemolytic, and because the non-secreted exotoxins remain in the swine immune system, a protective or immunogenic response is not developed. The recognition of the target cells is accomplished by one of two mechanisms: The first mechanism is the exotoxin recognition of a glycophorin receptor on the target cell membrane. A successful interaction with the cell membrane involves acylation in the active exotoxin. Accordingly, non-activated exotoxins are non-haemolytic, and since the exotoxins are not fixed to the target cells, the exotoxins provide a low immune response. The second mechanism is the exotoxin formation of a pore on the target cell membrane. This action is carried out by the three hydrophobic or transmembrane domains found in the N-terminal region of the exotoxins.

Dr. Pinol explained that the present invention is directed to developing an efficient vaccine against swine APP. In doing so, the exotoxins could not be removed from APP since an immune response against the exotoxin is needed to protect the animals from future APP infections. Similarly, non-secreted exotoxins or non-activated toxins cannot be expected to promote an efficient immune protection. However, the present inventors surprisingly found that by performing unmarked modifications in the APP genome (slide 5 of presentation), for example, deletion mutation of the coding sequence of a single hydrophobic domain in ApxI from a wild type serotype I strain (slide 4 of presentation), the modified ApxI could be exported outside APP in its native form, while the haemolytic activity of the recombinant strain was greatly reduced. Further analyses revealed that the modified ApxI was virtually non-haemolytic and nearly full immunogenic. The present inventors also deleted the second hydrophobic domain in ApxIIa exotoxin (slide 6 of presentation). The recombinant strain with deleted transmembrane regions in the apxIA and apxIIA was also non-haemolytic and immunogenic and both modified exotoxins were found exported outside of APP. The resulting recombinant strains were completely avirulent when tested on swine and effectively protected the animals when infected with a wild type strain of APP. Thus, deletion mutations in the transmembrane domain in both the apxIA and/or the apxIIA provided a safe and effective vaccine to protect swine against APP infections.

Mr. Pi then distinguished the claimed invention over the cited art to Reimer, MacInnes, and Prideaux. The claimed invention was distinguished over Reimer which disclosed 4 strains of APP strains.

1) Wild strain J45 (slide 8 of presentation 8): high immunogenic and strong haemolytic because activated ApxI and ApxII exotoxins are secreted (ApxI and ApxII secreted).

2) mIT4-H: chemical mutant (slide 10 of presentation page 10 in which the red colour indicates deletion): the whole operon *apxICABD* is deleted by mutagenesis. That means that *apx*IC, *apx*IA, *apx*IB and *apx*ID genes are completely deleted This strain is non-immunogenic and non-haemolytic (no secretion of ApxI and ApxII).

3) mIT4-H/pJFF801 (slide 12 of presentation page 12 in which the red colour indicates deletion and blue colour indicates restoration): *apx*IC and *apx*IA genes are completely deleted, and therefore ApxI exotoxin is not produced, whereas ApxII is produced, activated and secreted. This strain is low immunogenic and weak haemolytic (ApxI not expressed, ApxII secreted).

4) mIT4-H/pJFF800 (slide 13 of presentation page 13 in which the blue colour indicates restoration): activated ApxI and ApxII exotoxins are produced, as in wild type strain. This strain is high immunogenic and strong haemolytic (ApxI and ApxII are secreted).

Thus, Reimer does not disclose the presently claimed mutation in the transmembrane domain of *apx*IA gene or in a transmembrane domain of *apx*IIA gene.

The claimed invention was distinguished over MacInnes which only discloses mutations in the transport genes (B and/or D) to obtain cell-associated toxins. The APP strains of MacInnes produce an RTX toxin that is substantially cell associated, i.e., the toxin is not secreted because the B and/or D transport genes are inactivated (columns 13 and 14 of MacInnes). Also, MacInnes assess that transposon mutants with insertions in the *apx*IBD genes export little or no RTX and contain cell-associated RTX (column 30,

lines 44-46 of MacInnes). Further, MacInnes discloses that outer membrane proteins of *Actinobacillus pleuropneumoniae* can be altered by changing growth conditions (see column 21 and 22, Example 1), and that the quantity of cell-associated RTX toxin produced in culture is affected by the growth medium (column 23, lines 10-13). These teachings were pointed out to be closely related to the *E. coli* bacterial preparations disclosed in Example 3 of MacInnes.

The claimed invention was distinguished over Prideaux which discloses (1) inactivation of the RTX C gene in order to prevent activation of the structural RTX A gene (slide 17 of presentation), (2) inactivation of transport genes (RTX B or D genes) which are responsible for the secretion of RTX toxins (this is similar to MacInnes) (slide 19 of presentation), (3) partial or full inactivation of RTX A and RTX C genes (slide 21 of presentation), and (4) introduction of a functional RTX A gene into a microorganism which is incapable of producing an activated RTX toxin (slide 22 of presentation). Thus, Prideaux does not teach a mutation in one region of *apx*IA gene and with or without a mutation in one region of *apx*IIA gene.

Thus, the present inventors have found that the introduction of at least one mutation (e.g., deletion) in a transmembrane domain of the *apxIA* gene, with or without a mutation (deletion) in a transmembrane domain of the *apxIIA* gene surprisingly resulted in (slides 25 and 26 of presentation):

- maintenance of the structure of ApxI and ApxII exotoxins,

- secretion of the ApxI and ApxII exotoxins,

- non-haemolytic activity,

- immunogenicity and

- immunoprotective characteristics.

Supervisor Mondesi appeared to agree with Applicants' explanation and commented that it appeared the novelty of the claimed invention rests in the modification to *apx*IA gene to produce the claimed immunogenic and non-haemolytic mutant, and that the sequences between *apx*IA gene and *apx*IIA gene appeared to be relatively conserved. Supervisor Mondesi mentioned that it appeared the *apx*IA gene is not completely deleted in the present invention. Accordingly, Supervisor Mondesi believed the arguments were sufficient to address the prior art rejections, and Reimer with regard to the Restriction Requirement.

Applicants' representative also proposed claim amendments to help address the §112, second paragraph issues, and the Supervisor stated that the proposed claim amendments should be sufficient to overcome the §112 second paragraph rejections, and place the claims in condition for allowance. Specifically, Applicants indicated that claim 13 would be amended to replace "optionally" with "with or without", and SEQ ID NOs. will be included to identify *apx*IA and *apx*IIA to address the §112, second paragraph rejections.

Supervisor Mondesi also suggested amending withdrawn claim 24 (from which claims 25-29 directly or indirectly depend) in the same manner to rejoin the withdrawn method claims. Supervisor Mondesi also suggested amending withdrawn claims 18, 27 and 29 in the same manner as proposed for rejoinder. With regard to withdrawn claims 20 (from which claim 21 depends) and claim 22 (from which claim 23 depends), Supervisor Mondesi mentioned that if the

requirements for the deposits are met, i.e., the 112 requirements are satisfied, and additional searching does not produce any further art, then he believes claims 21 and 22 may also be rejoined.

It is respectfully submitted that the instant STATEMENT OF SUBSTANCE OF INTERVIEW complies with the requirements of 37 C.F.R. §§1.2 and 1.133 and MPEP §713.04.

It is believed that no petition or fee is required. However, if the USPTO deems otherwise, Applicant hereby petitions for any extension of time which may be required to maintain the pendency of this case, and any required fee, except for the Issue Fee, for such extension is to be charged to Deposit Account No. 19-4880.

Respectfully submitted,

/Tu A. Phan/

Tu A. Phan, Ph.D.
Registration No. 59,392

SUGHRUE MION, PLLC
Telephone: (202) 293-7060
Facsimile: (202) 293-7860
WASHINGTON OFFICE
23373
CUSTOMER NUMBER

Date: July 14, 2009